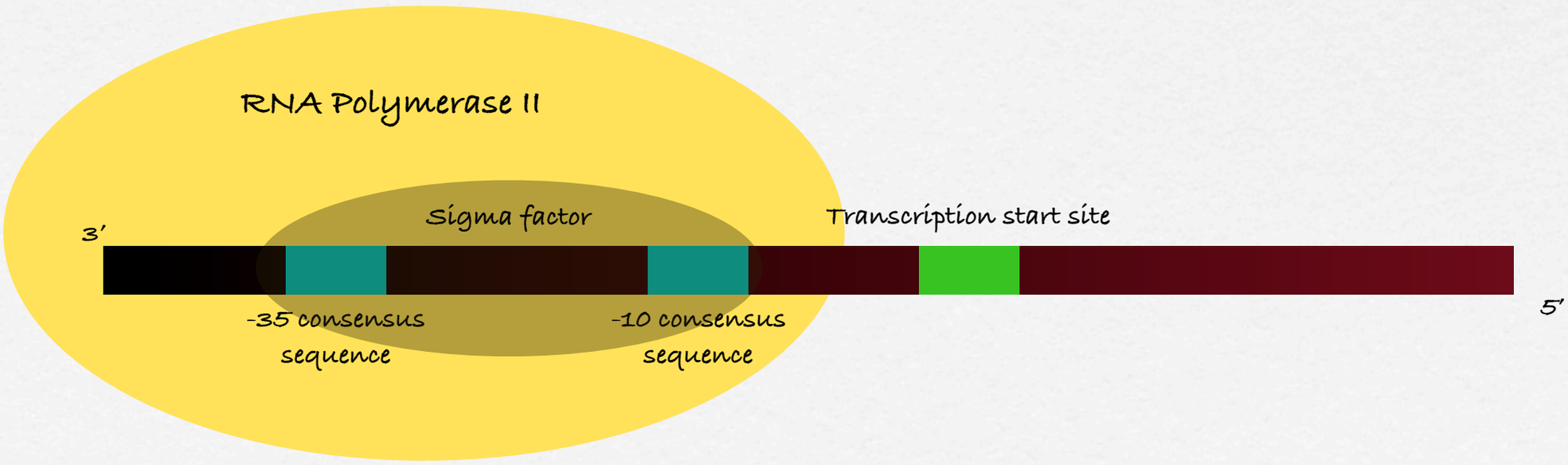
Synthetic Biology for

iGEM

Promoter Regions

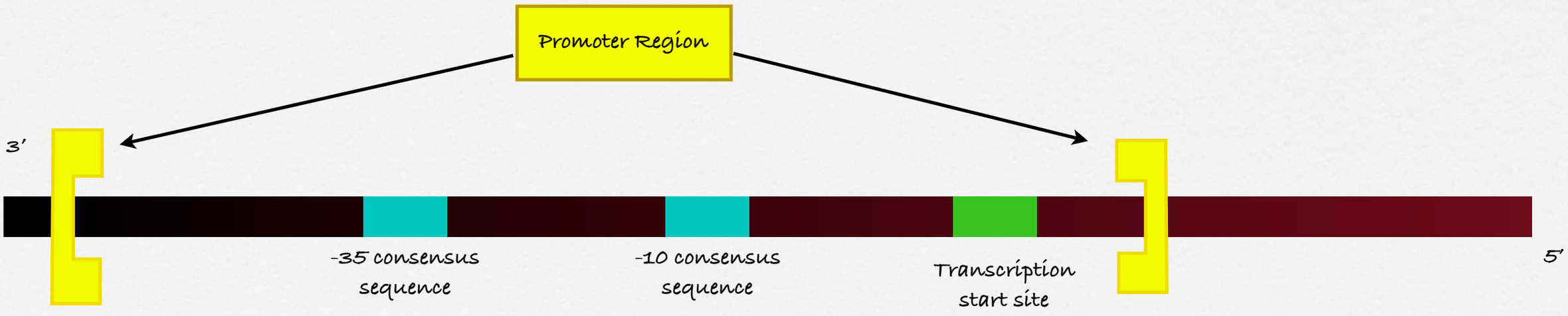
Recall: Transcription Initiation

- The first step of transcription (converting DNA to RNA) is the binding of RNA polymerase
- RNA polymerase is "recruited" to bind by the binding of transcription factors
- In E. Coli, the main transcription factor is the sigma factor



The Promoter Region

- The promoter region is the area upstream of the gene it promotes
- In E. coli the promoter is typically small (<300 bp)
- The promoter region refers to the RNA polymerase binding site and everything that affects binding (activators, repressors, etc.)

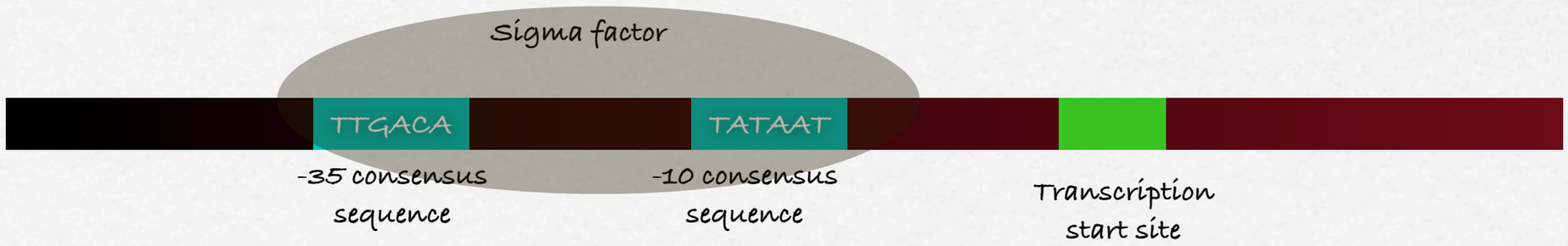


Core Promoter

- The core promoter is the minimal region necessary to initiate transcription
- In bacteria, this contains three elements: the Transcription Start Site, -10 consensus sequence, and -35 consensus sequence

The Consensus Sequences

- The -10 and -35 consensus sequences are two regions of DNA that the sigma factor binds to
- The sigma factor then recruits RNA polymerase, and transcription begins
- Different sigma factors recognize different sequences



Consensus Sequence?

- Most -10 and -35 sequences have at least one difference from their consensus sequence
- "Consensus sequence" refers to the sequence of bases with the highest likelihood of existing
- Few promoters use the consensus sequence in full, and those that do don't work well

-10

TATAAT

for -10 sequence

T	A	T	A	A	T
77%	76%	60%	61%	56%	82%

-35

TTGACA

for -35 sequence

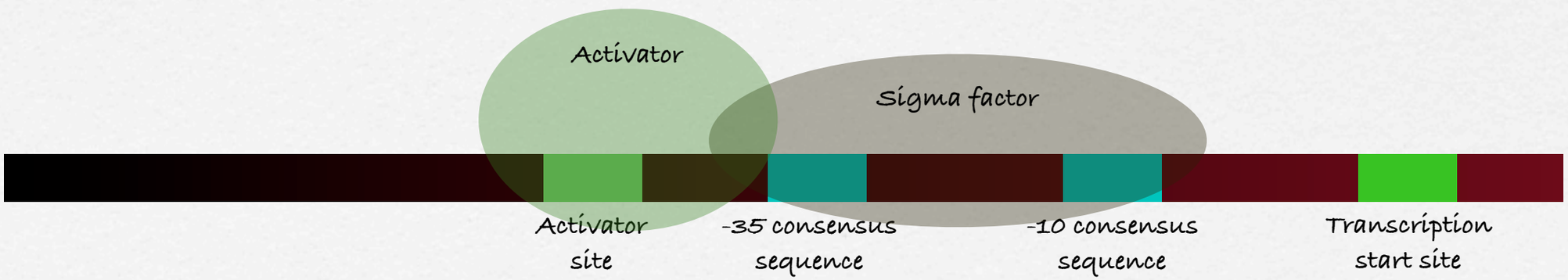
T	T	G	A	C	A
69%	79%	61%	56%	54%	54%

Other Elements of the Promoter

- Aside from the core promoter, other, non essential elements exist
- These elements determine when a gene should be transcribed, including how often
- Types of other elements include regions that activators or repressors may bind to

Activators

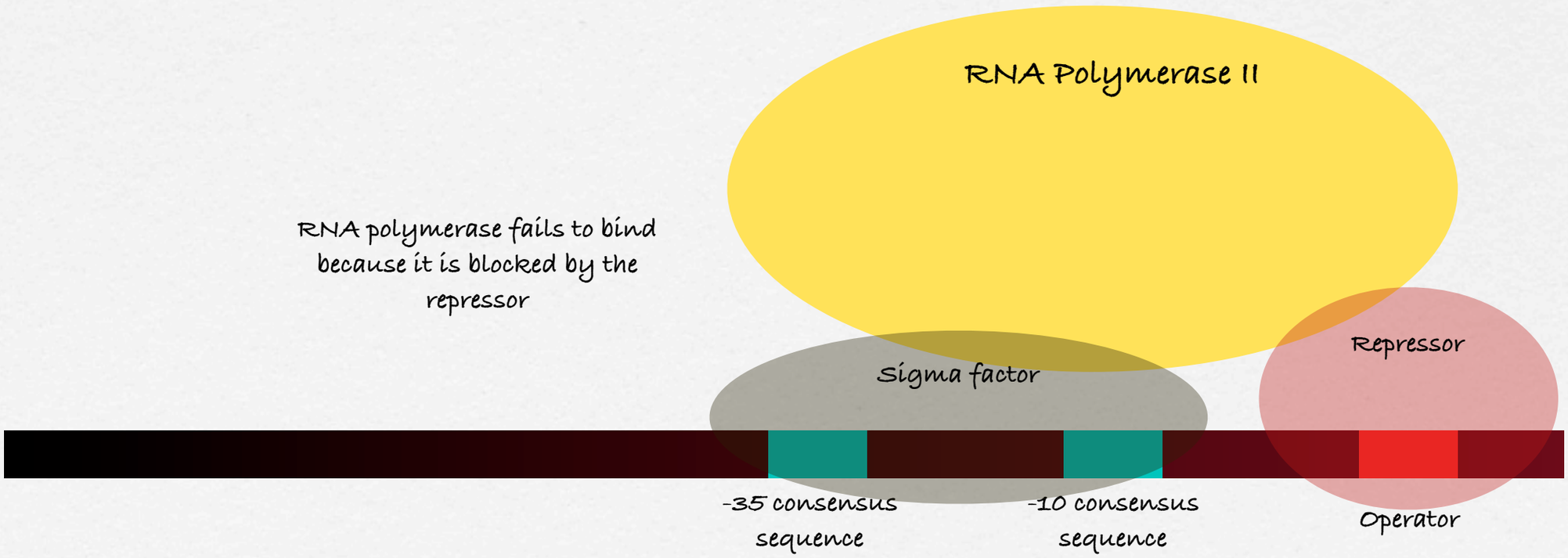
- Activators are proteins that bind the DNA upstream of the promoter
- Activator proteins interact with transcription factors to stabilize binding
- The DNA region the activator binds to is called the activator site



The activator binds to the activator site, and has positive interaction with the transcription factor (sigma factor). This stabilizes the sigma factor, keeping it bound to the consensus sequences longer so that RNA polymerase can be recruited.

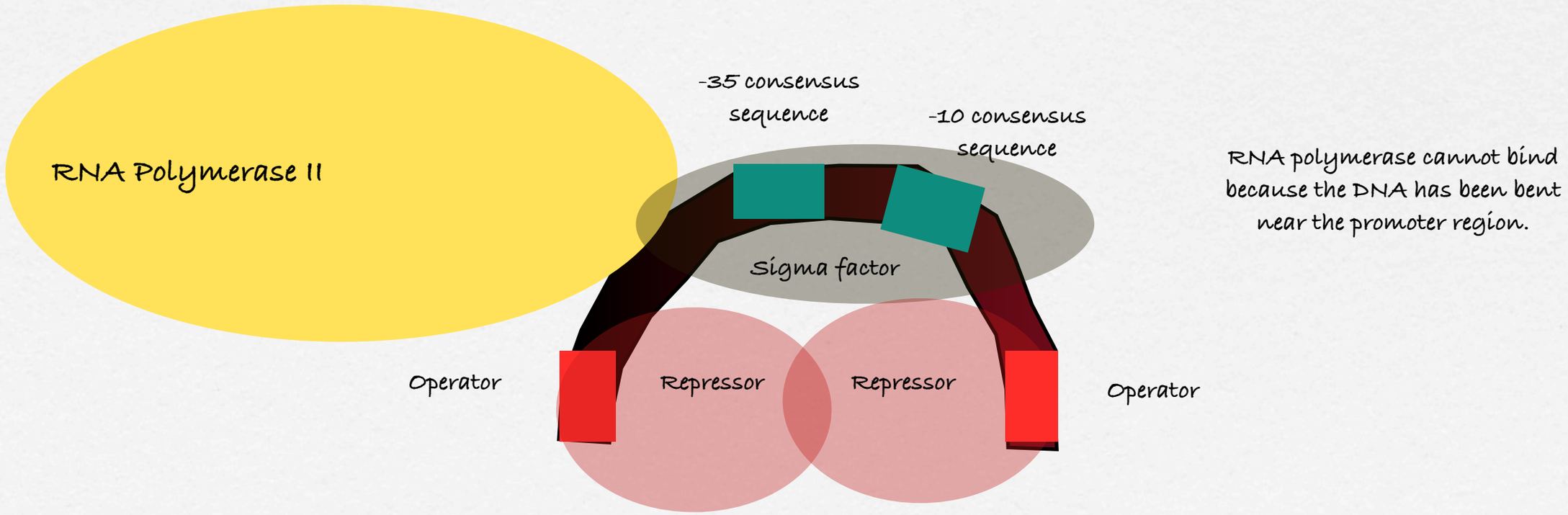
Repressors

- Repressors bind the DNA, usually downstream of the promoter
- Repressors inhibit the binding of RNA polymerase, blocking transcription
- The DNA site a repressor binds is called an operator



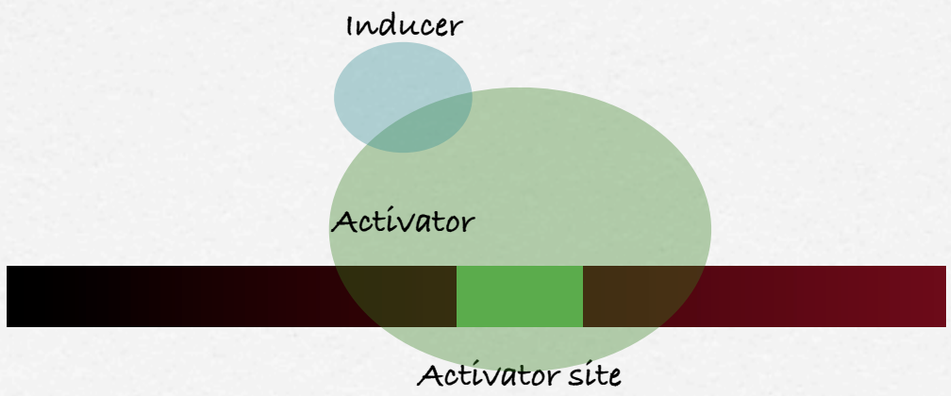
Repressors II

- Many repressors do not directly inhibit RNA polymerase
- Often, they have an operator before and after the promoter region
- Two repressors bind and dimerize, creating a fold in the DNA that prevents binding

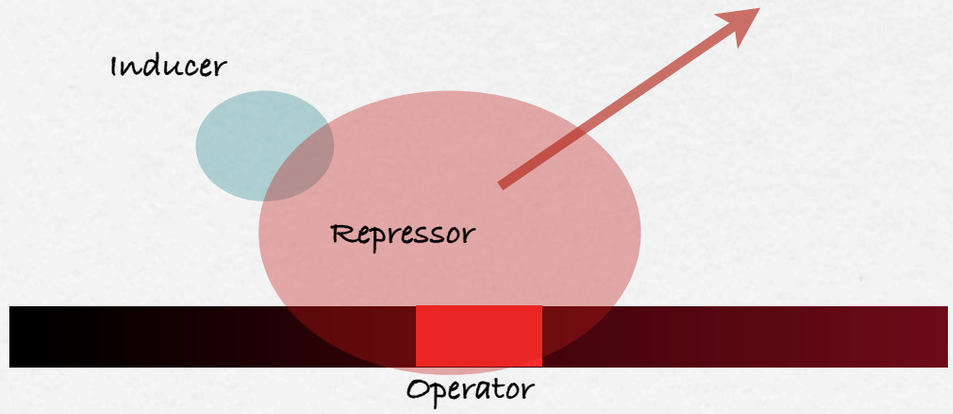


Inducers

- Inducers are small molecules (small proteins, carbohydrates, etc.) that bind activators or repressors
- Inducers will help stabilize activators
- Inducers will remove repressors from operators
- Allolactose is an inducer for the lac operon system



The inducer stabilizes the activator, strengthening the chance of transcription



The inducer detaches the repressor from the DNA, allowing transcription

Operons

- Operons are groups of genes that are regulated by a single promoter
- When the promoter is induced, all the genes in the operon are transcribed at once
- This creates faster response to environmental changes

Example: Lac Operon

Glucose

When only glucose is present, the lac operon is off. Glucose is the primary food source of the cell.

Glucose + Lactose

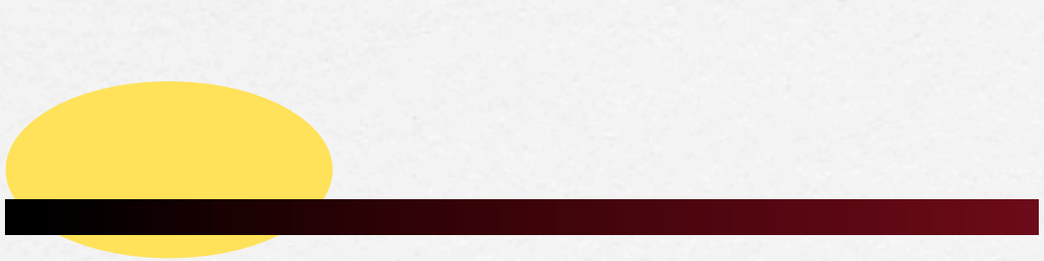
Glucose represses the lac operon. In the presence of both lactose and glucose, the system is off because the cell gets nutrients from glucose.

Lactose

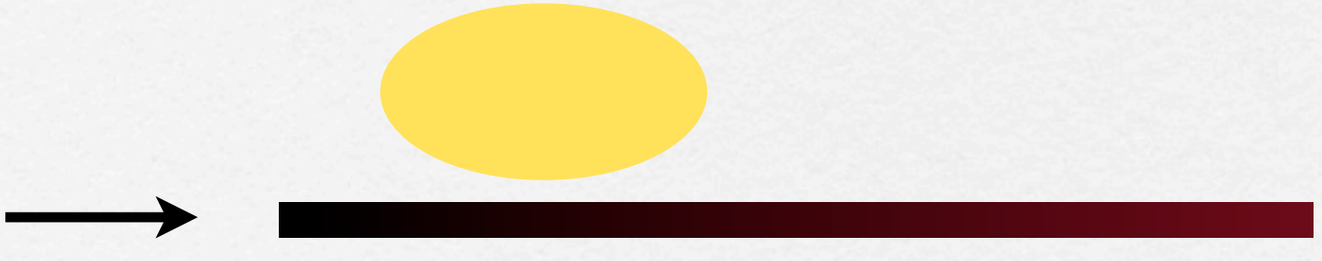
When lactose alone is present, the lac operon is activated. The lac operon expresses proteins that digest lactose, allowing the bacteria to use lactose as the primary food source.

A Quick Note on Rates...

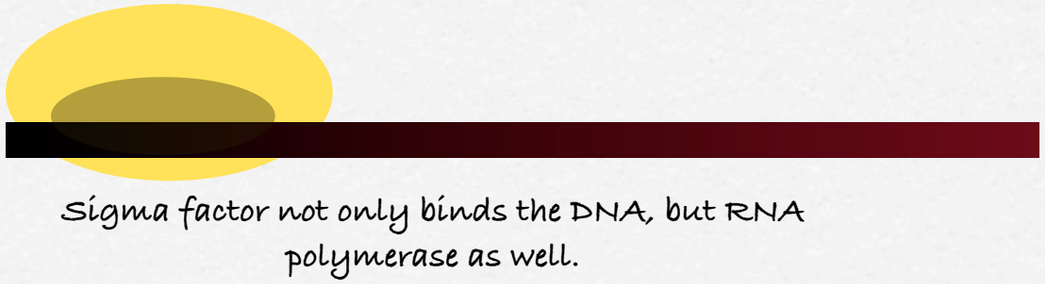
- Biology is a constantly moving process
- Processes like DNA binding are weak, and may break apart (dissociate) as quickly as they are formed
- When lots of proteins bind together, the chances of dissociating decrease



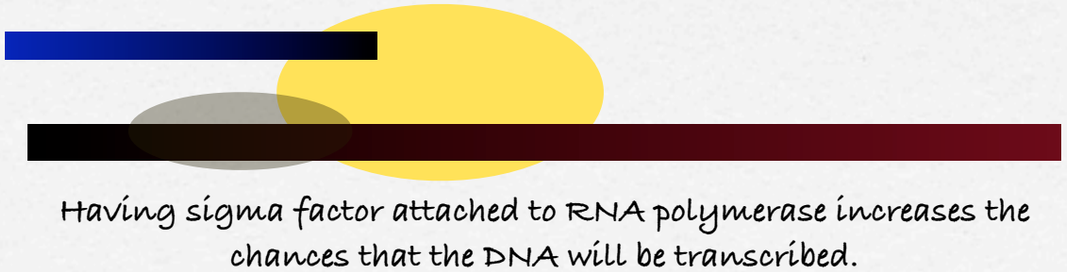
RNA Polymerase II only binds weakly to DNA.



Before it can transcribe anything, it will likely dissociate from the DNA.

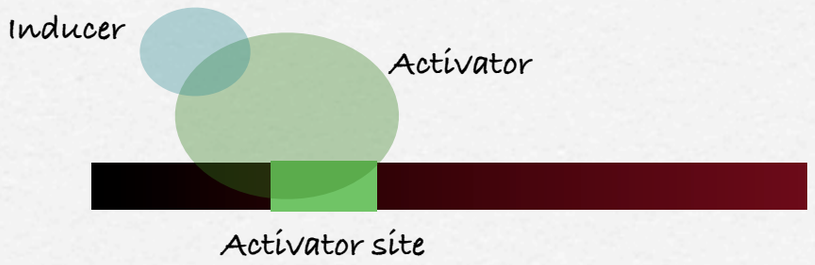


Sigma factor not only binds the DNA, but RNA polymerase as well.

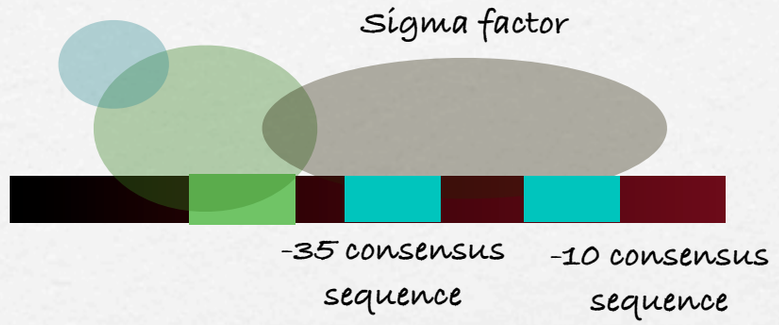


Having sigma factor attached to RNA polymerase increases the chances that the DNA will be transcribed.

Rates and Promoters

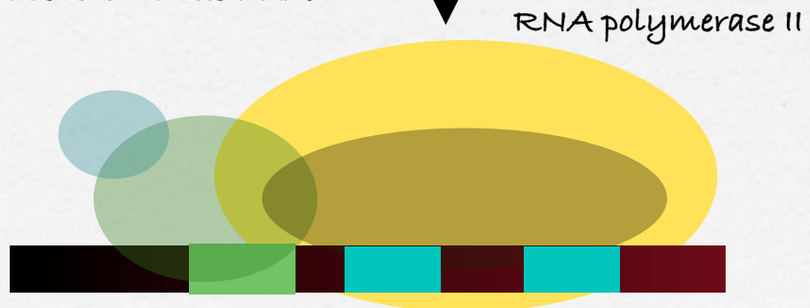


The inducer stabilizes the activator binding to the DNA



The activator stays bound to the DNA long enough to recruit the sigma factor to bind the consensus sequences.

If the sigma factor binds the DNA long enough, it will recruit an RNA polymerase to bind the DNA.



If RNA polymerase II is stable, it will begin transcription.

Important Terminology

Transcription: The process of converting DNA into RNA (usually mRNA). This is the first step in generating protein.
RNA polymerase II: The primary enzyme responsible for transcription.
Promoter Region: The section of DNA, before a given gene, that is responsible for the binding of transcription factors and RNA polymerase.
Transcription Factors: DNA binding proteins. Transcription factors interact with RNA polymerase, stabilizing its binding to DNA to start transcription. Transcription factors in bacteria bind the DNA at the consensus sequences.
Sigma Factor: The principle transcription factor for E. coli. There are seven known sigma factors in E. Coli, but the principle factor is sigma 70 (Named because it has a molecular weight of 70 kiloDaltons (70,000 grams/mole))
Consensus Sequence: Sigma factor binding regions in the promoter region. The term consensus sequence refers to the sequence not being the same for every promoter. The given consensus sequences are based on base pairs with the highest occurrence. In E. Coli, the consensus sequences are found at -10 and -35 (10 and 35 base pairs before the transcription start site)
Transcription Start Site: The beginning of RNA transcription. Downstream of binding sequences.
Activator: A protein that binds DNA and stabilizes the binding of transcription factors.
Activator Site: The region of DNA an activator binds to.
Repressor: A protein that binds DNA and destabilizes the binding of transcription factors/RNA polymerase.
Operator: The region of DNA a repressor binds to.
Inducer: Promotes transcription by either stabilizing an activator or destabilizing a repressor.
Operon: A set of genes under the regulation of the same promoter. They are transcribed at once.
Lac Operon: A famous operon in E. coli that operates in the presence of lactose and absence of glucose. The lac operon creates proteins that digest lactose to be used as the main source of energy for cells.